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Neoplastic Involvement of Granulocytic Lineage, Not Granulocytic-Monocytic, Monocytic, or Erythrocytic Lineage, in a Patient With Chronic Neutrophilic Leukemia

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Chronic neutrophilic leukemia (CNL) is a very rare myeloproliferative disorder. To determine the neoplastic origin of CNL, morphological and cytogenetical studies were made of colonies derived from hematopoietic progenitors of a patient with CNL. The patient's hematopoietic progenitors spontaneously formed colonies consisting of mature granulocytes, and cytogenetical study of the colonies indicated chromosome abnormalities identical to those in the patient's bone marrow cells. Analysis of colonies consisting of granulocytes and macrophages, macrophages, or erythrocytes disclosed a normal karyotype. These results demonstrated that the neoplastic process in this patient with CNL originated in hematopoietic progenitors capable of differentiating only into granulocytes, and not granulocytes and monocytes, monocytes, or erythrocytes. Am. J. Hematol. 57:221–224, 1998. © 1998 Wiley-Liss, Inc.

Key words: chronic neutrophilic leukemia; neoplastic origin; hematopoietic progenitors

INTRODUCTION

Chronic neutrophilic leukemia (CNL) is a rare myeloproliferative disorder and very few cases have been reported. The diagnosis of CNL is based on the persistent unexplained neutrophilia, hepatosplenomegaly, elevated neutrophil alkaline phosphatase (NAP), and absence of the Philadelphia (Ph^1) chromosome and rearrangement of the major breakpoint cluster region (M-bcr) [1–4]. The origin of the neoplasia has been considered to be granulocyte-committed progenitors [1,5,6], but this hypothesis remains to be validated. Here we describe a patient with CNL, in whom the neoplastic origin was confirmed to be granulocyte-committed progenitors by clonal cell assay and cytogenetical analysis.

CASE REPORT

The patient, a 63-year-old Japanese man, was admitted to Ehime University Hospital because of abdominal fullness in September 1994. Physical examination on admission indicated massive hepatosplenomegaly. The laboratory test results were as follows: Hb 7.0 g/dL, white blood cell (WBC) count 84×10^9/L with 92.0% neutrophils and 0.5% myeloblasts, and platelet count 30×10^9/L. The neutrophilia was not associated with a shift to the left, and there was no evident eosinophilia or basophilia. NAP score was elevated and the level of serum-granulocyte colony-stimulating factor (G-CSF) was within the normal limits. Bone marrow aspiration revealed granulocytic hyperplasia with normal maturation and marked decrease of erythroid cells and megakaryocytes. The cells showed no evident dysplastic features. There was no Ph^1 chromosome and no rearrangement of the M-bcr region upon Southern blotting. In the bone marrow cells, RT-PCR amplification did not yield bcr-abl fusion transcripts. On the basis of these data, the patient was diagnosed as having CNL, and treatment with hydroxyurea and 6-mercaptopurine (6-MP) was

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started. Leukocytes decreased from 84 × 10^9 to 21 × 10^9/L and no signs of blastic transformation were seen. This chemotherapy, however, worsened the thrombocytopenia (5 × 10^9/L), and in October 1994, the patient died from uncontrollable intracerebral hemorrhage. Autopsy indicated that the bone marrow, spleen, and liver had been affected by diffuse proliferation of granulocytic cells.

MATERIALS AND METHODS

Cell Culture

Bone marrow and peripheral blood mononuclear cells (BMMNCs and PBMNCs) were separated by centrifugation on a Ficoll-Conray gradient. The MNCs were suspended in RPMI 1640 medium containing 10% fetal calf serum (FCS, GIBCO, Grand Island, NY) in plastic culture dishes and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for 2 hr. Then, non-adherent MNCs were depleted of T cells by sheep red blood cell rosetting, and 1 × 10^5 non-adherent and non-T MNCs were cultured in α-medium containing 0.8% methylcellulose, 20% FCS, and 1% bovine serum albumin (BSA) with or without various cytokines in a 35-mm tissue culture dish at 37°C for 14 days.

Morphological Examination of Colonies

Individual colonies were removed from the culture and placed in PBS containing 5% FCS. Each cell suspension was centrifuged in a Shanden Cytospin and stained with May-Grünwald-Giemsa solution.

Cytogenetical Analysis

The karyotypes of 30 selected colonies of each type, patient's bone marrow cells, phytohemagglutinin (PHA)-stimulated patient's PBMNCs, and an EB-transformed B-cell line derived from PBMNCs were analysed by the trypsin-Giemsa banding method. Twenty metaphases were analyzed for each sample. For cytogenetical analysis of the colonies, 1 × 10^4 cells were cultured in a 35-mm culture dish to avoid cell contamination from neighboring colonies.

RESULTS

Colony Formation and Analysis of Cell Composition

The patient's PBMNCs and BMMNCs formed colonies without cytokines (Table I). All of the spontaneous colonies were composed of a large number of mature granulocytes and several intermediate or immature cells. G-CSF markedly enhanced granulocyte (G)-colony formation. Colonies formed by granulocyte-macrophage (GM)-CSF or interleukin-3 (IL-3) were essentially G-colonies and the rest were GM-colonies or macrophage (M)-colonies (Fig. 1). The cells formed a few colonies from burst-forming units in erythrocytes (BFU-E) after addition of erythropoietin (EPO) and hardly formed megakaryocyte colonies in response to thrombopoietin (TPO) (Table I). No G-CSF activity was detected in the supernatants of the patient's non-adherent and non-T PBMNCs or BMMNCs (data not shown).

TABLE I. Colony Formation of the Patient's Cells*

Cytokine	PB No. of colonies/ 1 × 10^5 cells	BM No. of colonies/ 1 × 10^5 cells
(−)	127 ± 35	179 ± 42
G-CSF (100 U/ml)	597 ± 79	725 ± 139
GM-CSF (100 U/ml)	312 ± 70	592 ± 76
IL-3 (100 U/ml)	415 ± 54	506 ± 89
EPO (2 U/ml)	0	13 ± 2
TPO (10 ng/ml)	0	0

*Values are the means ± SD in triplicate cultures.

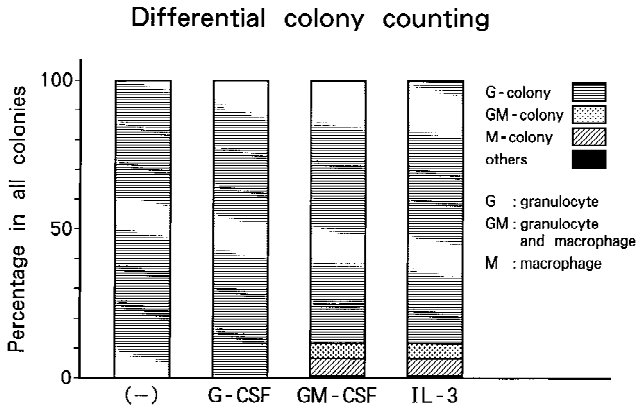

Fig. 1. Results of differential colony counting. All of the spontaneous and G-CSF-induced colonies from the patient's BMMNCs were G-colonies. GM-CSF or IL-3-induced colonies were essentially G-colonies, and the rest were GM-colonies or M-colonies.

Cytogenetical Analysis of Colonies

Analysis of spontaneous and G-CSF-induced colonies demonstrated chromosome abnormalities identical to those in bone marrow cells: 46, XY, del(11)(q21), del (12)(p13) or 46, XY, add(1)(q21), del(6)(p23), add(7)(q22), add(7)(q32), del(11)(q21), del(12)(p13) (Fig. 2). G-colonies formed in response to GM-CSF and IL-3 showed the same chromosome abnormalities (data not shown). Analysis of the patient's GM-colonies, M-colonies, and BFU-E colonies demonstrated the normal karyotype: 46, XY (Table II). PHA-stimulated T-lymphocytes and the EB-transformed B-cell line also showed the normal karyotype (data not shown).

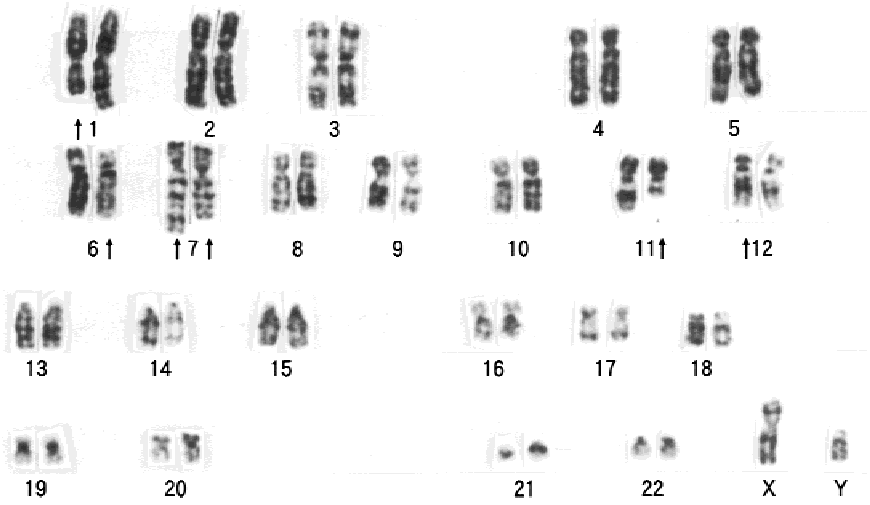

Fig. 2. Cytogenetical analysis of the patient's spontaneous colonies. 46, XY, add(1)(q21), del(6)(p23), add(7)(q22), add(7)(q32), del(11)(q21), del(12)(p13).

TABLE II. Cytogenetical Analysis*

Karyotype	BM cells	G-colonies G-CSF (−)	G-colonies G-CSF (+)	GM-colonies	M-colonies	BFU-E colonies
46, XY, add(1)(q21), del(6)(p23), add(7)(q22), add(7)(q32), del(11)(q21), del(12)(p13)	17	12	15	0	0	0
46, XY, del(11)(q21), del(12)(p13)	3	8	5	0	0	0
46, XY	0	0	0	20	20	20

*20 metaphases were analysed on each sample.

DISCUSSION

Since the first description of CNL in 1920 [7], more than 30 cases have appeared in the literature. It has been suggested that CNL may possibly constitute a distinct clinicopathologic subgroup within the myeloproliferative disorders [1,5,6]. However, it is sometimes difficult to distinguish CNL from Ph[1]-negative CML or chronic myelomonocytic leukemia (CMMoL) [8]. Studies on the neoplastic origin of these diseases should provide the means for distinguishing them. CML is defined as neoplastic involvement of pluripotent progenitors and characterized by increased colony-forming units in granulocytes, erythrocytes, macrophages, and megakaryocytes (CFU-GEMM) [9]. CMMoL has been reported to show an increase of CFU-GM progenitors that have acquired an IL-6-mediated autocrine system [10,11]. CNL may be produced as a result of the neoplastic involvement of granulocyte-committed progenitors [1,5,6], but this hypothesis remains to be confirmed. Therefore, in this study, morphological and cytogenetical studies were done on colonies derived from hematopoietic progenitors of a patient with CNL showing chromosome abnormalities. Cytogenetical analysis of cells from CNL patients usually demonstrates a normal karyotype, but in a few cases, various chromosome abnormalities have been described [12,13]. Our patient had chromosome abnormalities without the Ph[1] chromosome. The stage of neoplastic differentiation could thus be identified by cytogenetical analysis of colonies from hematopoietic cells of different differentiation stages. The patient's PBMNCs and BMMNCs spontaneously formed colonies consisting of a large number of mature granulocytes and several intermediate or immature cells, and chromosome analysis of the spontaneous colonies showed chromosome abnormalities identical to those in the bone marrow cells. G-CSF enhanced G-colony formation and the G-colonies also demonstrated the same chromosome abnormalities. Cytogenetical analysis of the GM-colonies and M-colonies formed by exposure to IL-3 or GM-CSF and BFU-E colonies induced by EPO showed a normal karyotype. PHA-stimulated T-lymphocytes and EB-transformed B-cells also exhibited a normal karyotype. Thus it appears that the neoplastic process in our patient originated in progenitors capable of differentiating into granulocytes, but not granulocytes and monocytes, monocytes, erythrocytes, or lymphocytes, and that some hematopoietic progenitors acquired the ability to differentiate into granulocytes spontaneously in vitro. To our knowledge, this is the first report providing evidence for the neoplastic origin of CNL. Contrary to our findings, several reports have suggested that the neoplastic origin of CNL appears to be pluripotent progenitors, based on

observations of CNL in patients with polycythemia vera [14,15] and abnormal platelet functions in patients with CNL [5,16]. However, there have been no reports focusing on the hematopoietic progenitors and providing direct evidence of the neoplastic origin of CNL.

Unfortunately, we could not study the CD34 positive progenitors of the patient, because we had spent his bone marrow cells. Further investigators who may deal with similar patients should isolate CD34 positive progenitors from their bone marrow cells and see if the chromosome lesions are present in earlier progenitors.

We have reported a patient with CNL in which only granulocyte-committed progenitors, but not other progenitors, were involved in the neoplastic process. We are convinced that similar studies of further cases will clearly demonstrate CNL to be a distinct clinicopathologic subgroup within the myeloproliferative disorders.

REFERENCES

1. Nisinari T, Takahashi T, Miura A: Chronic neutrophilic leukemia. Clinicopathological investigation on Japanese cases with the questionnaire. Jpn J Clin Hematol 30:45–50, 1989.
2. Zittoun R, Rea D, Hoang Ngoc L, Ramond S: Chronic neutrophilic leukemia: A study of four cases. Ann Hematol 68:55–60, 1994.
3. Kwong YL, Cheng G: Clonal nature of chronic neutrophilic leukemia. Blood 82:1035–1036, 1993.
4. Lugassy G: Author's reply. Am J Hematol 35:140, 1990.
5. Dotten DA, Pruzanski W, Wong D: Functional characterization of the cells in chronic neutrophilic leukemia. Am J Hematol 12:157–165, 1982.
6. Mehrotra PK, Winfield DA, Fergusson LH: Cellular abnormalities and reduced colony forming cells in chronic neutrophilic leukemia. Acta Haematol 73:47–50, 1985.
7. Tuohy EL: A case of splenomegaly with polymorphonuclear neutrophil hyperleukocytosios. Am J Med Sci 160:18–25, 1920.
8. Kantarjian HM, Jurzrock R, Talpaz M: Philadelphia chromosome-negative chronic myelogenous leukemia and chronic myelomonocytic leukemia. Hematol Oncol Clin North Am 4:389–404, 1990.
9. Kohsaki M, Hara H: Hemopoietic stem cells in chronic myelogenous leukemia: Abnormalities and behavior. Acta Haematol Jpn 46:1589–1597, 1983.
10. Akashi K, Shibuya T, Harada M, Takamatsu Y, Uike N, Eto T, Niho Y: Interleukin-4 suppresses the spontaneous growth of chronic myelomonocytic leukemia cells. J Clin Invest 88:223–230, 1991.
11. Yanagisawa K, Hatta N, Watanabe I, Horiuchi T, Hasegawa H, Fujita S: IL-4 stimulates the growth of chronic myelomonocytic leukemia cells (CMMoL) once leukemic transformation has occurred. Leukemia 9:1056–1059, 1995.
12. Di Donate C, Croci G, Lazzari S, Scarduelli L, Vignoli R, Buia M, Tramaloni C, Maccari S, Plancher AC: Chronic neutrophilic leukemia: Description of new cases with karyotypic abnormalities. Am J Clin Pathol 12:157–165, 1982.
13. You W, Weisbrot I: Chronic neutrophilic leukemia. Report of 2 cases and review of the literature. Am J Clin Pathol 72:233–241, 1979.
14. Lugassy G, Farhi R: Letter to the editor: Chronic neutrophilic leukemia associated with polycythemia vera. Am J Hematol 31:300–301, 1989.
15. Lurlo A, Foa P, Maiolo AT, Luksch R, Capsoni F, Polli EE: Letter to the editor: Polycythemia vera terminating in chronic neutrophilic leukemia: Report of a case. Am J Hematol 35:139, 1990.
16. Mori H, Ikegami T, Shimizu T, Harada H, Miyoshi Y, Okada S, Takizawa Y, Nikura H, Terada H, Fijita K: Changes of the megakaryocyte-platelet system in chronic neutrophilic leukemia. Jpn J Clin Hematol 32:1574–1576, 1991.